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WAYS OF INCREASING THE GAS RESISTANCE OF PLANTS

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Pollution of the atmosphere with various gases, dust, and aerosols, which are copiously discharged by industrial enterprises, creates extremely unfavorable conditions for planting greenery in their territory and for raising agricultural crops. Under these conditions, plants suffer a sharp loss in their productivity and decorative nature, and often die.

According to modern concepts, a toxic gas entering via the stoma or epidermis into the internal portion of the leaf dissolves in the water of the cellular membranes and reacts with the cytoplasm. The underlying cells of the stomal cavities are the first to be affected. Following their plasmolysis and wrinkling of the cellular membranes, the gas penetrates the deeply situated cells of the spongy and palisade parenchymas [12, 17].

Being dissolved in water, the gas either forms an acid or an alkali. The latter enters into reaction with the cellular contents,

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*The Central Republican Botanical Garden of the Academy of Sciences of the Ukrainian SSR.

and part of them are neutralized by the corresponding bases or acids, while a part remains in the free state. The acids cause destruction of the chlorophyll, reduction in the pH of leaf tissues, reduce the stability of the biocolloids of the cytoplasm, and increase its general oxidizability [3]. Moreover, there is an increase in the dispersion of colloids and the hygroscopicity of the tissues, which leads to elevation of cuticular transpiration [13], a drop in turgor, disorder of the enzymatic apparatus, and to disruption of the basal metabolism within cells of the leaf and the transport systems [14].

The disruption of photosynthetic activity of plants that have been exposed to gases varies. A high concentration of the toxic gas causes cessation of photosynthesis almost instantaneously. Regions of the leaf undamaged by the gas gradually restore photosynthesis to the level of the ungassed leaves, or even exceed it. On this basis, Thomas [19] considers that gas injury of leaves of up to 5% of their area does not reduce the harvest. The effect of toxic gases on the intensity of respiration seems less clearly defined.

The harmful effect of gas in the same concentration appears variously, depending upon the species of plant, weather conditions, and the age of the leaf. There are species, as well as specific individuals within a single species, that are distinguished by increased gas resistance. The gas acts destructively on the middle-aged leaves, which are characterized by the most intensive gas exchange. From the cited data, one may conclude that the toxic gas that has penetrated the tissue of the leaf causes profound disruption of the basal metabolism and unregulated loss of water, which leads to its suppression or death.

Among the many problems related to the gas resistance of plants, we gave our main attention to clarifying the causes of differing degrees of resistance of individual species and of individuals to the toxic gases, to their harmless concentrations, and to their effect on photosynthetic activity.

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It was first necessary to clarify the course of accumulation of toxic doses of substances in leaves during their fumigation with sulfur dioxide and chlorine. As is known, sulfur and chlorine are vital elements of plant nutrition, although they are consumed in infinitesimal amounts. The comparative determination of the content of the referred elements was made in the leaves of plants grown at the Central Republican Botanical Garden of the Academy of Sciences of the Ukrainian SSR. Leaves exposed for 1 hour to SO_2 fumigation in a concentration of $15 \cdot 10^{-5}$, or to chlorine in a concentration of $5 \cdot 10^{-5}$% served as the experimental leaves, while the control were leaves of the same plants not exposed to gas. The accumulation of chlorine in the leaves of plants located on the territory of one of the enterprises, constantly containing an increased amount of chlorine was simultaneously determined. The chlorine content in samples was determined by the method of Delaville and Brown [2], while that of sulfur was determined by the method of K. N. Pochnik [6]. Some of the results obtained are given in Table 1.

The data of Table 1 indicate that each species of plants is characterized by a unique level of accumulation of chlorine in the leaves, fluctuating within limits of 0.1 - 0.5, and of sulfur — 0.2 - 0.9%, under ordinary conditions of growth location. Plants grown in the gassed atmosphere contain several times more chlorine in the leaves (1.0 - 2.25%). The slow accumulation of this element in plants does not cauce acute lesions. Necrosis of individual regions and of whole leaves occurs gradually, predominantly in the second half of the vegetation period.

With artificial fumigation, acute injury to the leaves appears more strongly, the greater the amount of toxic elements the plant accumulates in its assimilating organ per unit of time (for example, we shall compare the pine and the poplar with the olive and the birch, etc.). Additionally, a tendency toward a decrease in injury to leaves caused by the referenced gases clearly emerges proportional to the increase in accumulation of these elements in the plant prior to fumigation. This provides a basis to draw the general conclusion

TABLE 1. THE ACCUMULATION OF SULFUR AND CHLORINE (IN % ABSOLUTELY DRY WEIGHT) IN UNGASSED LEAVES (CONTROL), FUMIGATED LEAVES, AND LEAVES OF PLANTS TAKEN AT THE TERRITORY OF A PLANT

Variation of experiment	Control	Degree of damage, points	Content of chlorine, %	Degree of damage, points	Content of sulfur, %
Control	False acacia	-	0.15	-	0.23
Fumigated	" "	1	0.17	0	0.40
Control	Wych-elm	-	0.29	-	-
Fumigated	"	0	0.32	0	-
Plant territory	"	9	1.30	0	-
Control	Pedunculate oak	-	0.16	-	0.21
Fumigated	" "	0	0.17	0	0.35
Plant territory	" "	1	0.73	0	-
Control	Norway maple	-	0.36	-	0.22
Fumigated	" "	2	0.40	3	0.67
Plant territory	" "	10	2.25	-	-
Control	Russian olive	-	0.17	-	0.88
Fumigated	" "	0	0.20	0	0.90
Plant territory		0	0.63	-	-
Control	Scotch pine	-	0.09	-	-
Plant territory	" "	10	0.14	-	-
Control	Red poplar	-	0.08	-	0.32
Fumigated	" "	2	0.12	0	0.42
Control	Common ash	-	0.31	-	0.31
Fumigated	" "	8	0.40	0	0.39
Plant territory	" "	8	1.65	-	-
Control	Rose	-	0.40	-	0.59
Fumigated	"	8	0.50	0	1.03

that the degree of injury to the leaf during short-term gassing does not depend upon the absolute increment in content of the toxic element in it, but rather upon the relative increment.

On entering the leaf, the toxic gas not only causes local necrosis of individual regions of the mesophyll of the leaf, but also induces profound disruption of the internal processes of vital activity of the entire plant. An indicator of such disruption may be the change in intensity of its photosynthesis and respiration.

TABLE 2. CHANGE IN THE INTENSITY OF PHOTOSYNTHESIS AND RESPIRATION OF GASSED PLANTS (in mg CO_2 per 1 dm^2 per hour)

Kind	Measurement time, date	Fumigation variation	Degree of damage, points	Intensity	
				Photo-synthesis	Respira-tion
Apricot	Aug. 16-24, 11:00 a.m.	Chlorine	5	0.7	- 9.1
		Sulfur dioxide	0	5.9	- 5.1
		Control	-	7.0	- 3.6
"	Aug. 24, 1:00 pm.	Chlorine	5	7.5	-
		Sulfur dioxide	0	4.15	-
		Control	-	37.9	-
Walnut	Aug. 19 - Sept. 10, 12:00 m.	Chlorine	0	7.7	- 6.7
		Sulfur dioxide	0	14.6	- 13.3
		Control	-	29.6	- 8.1
"	Sept. 24 10:30 a.m.	Chlorine	0	2.2	- 5.7
		Control	0	4.8	- 3.3
Red poplar	Sept. 3, 12:00 m.	Chlorine	5	- 2.04	-
		Sulfur dioxide	0	3.5	-
		Control	-	35.2	-
"	April 9, 11:00 a.m.	Chlorine	5	- 13.2	-
		Sulfur dioxide	0	20.2	-
		Control	-	30.3	-
"	Sept. 14, 1:00 p.m.	Chlorine	5	- 1.9	-
		Sulfur dioxide	0	4.5	-
		Control	-	41.0	-
"	Sept. 23, 10:00 a.m.	Chlorine	0	7.3	-
		Sulfur dioxide	0	8.0	-
		Control	-	8.5	-
Mulberry	July 19, 11:30 a.m.	Chlorine	0	4.7	-
		Sulfur dioxide	0	9.5	-
		Control	-	5.3	-

For this purpose, the leaves were exposed to gassing according to the method described above. A comparative determination of the rate of gas exchange of leaves of identical age and under identical light before and after fumigation was carried out according to the method of Kh. N. Pochink [8]. The obtained data are given in Table 2.

The data of Table 2 shows that leaves, even those without visible damage caused by toxic gas, undergo decrease in the intensity of photosynthesis. The value of this decrease is not identical in the various species of plants. With the appearance of visible lesions of the leaves, the intensity of photosynthesis sharply drops, and occasionally the expiration of carbon dioxide occurs in place of its absorption.

The intensity of respiration of leaves exposed to the effect of a gas, as a rule, increases if the area of necrotic regions of the leaf is small (up to 20%). The increase in intensity of respiration is smaller in magnitude than the decrease in the rate of apparent photosynthesis. This indicates that the decrease in the rate of gas exchange occurs due to a drop in the intensity of pure photosynthesis. Our data confirm the conclusion already advanced by Stoklaza [18] regarding latent disruption of photosynthetic activity of plants exposed to the action of sulfur dioxide or chlorine.

In our experiments, coupling of inhibition of photosynthesis and injury to leaves noted by Thomas and other authors is not observed.

In species easily subject to gas injury, disruption of the chlorophyll occurs, as the result of which the total amount of chlorophyll in the unit mass of the leaf decreases by 15 - 30%. Simultaneously, the value of absorption by chlorophyll extracts in the region of 500 - 600 millimicrons change. There is a sharp drop in the photochemical activity of the chlorophyll and its bond with the protein--lipoid complex, resulting from the action of the toxic gas. The referenced facts show that SO_2 and Cl_2 have a destructive effect on the photosynthetic apparatus. This is one of the reasons for a drop in photosynthesis.

One can judge the course of change in pH, the oxidative-reduction (rH_2) potential and the activity of oxidative enzymes during penetration of the acid gases into the cells of the leaf on the

basis of the data of Table 3. Determination of pH was carried out using a pH meter, of catalase — by the iodometric method of Kh. N. Pochink [7], of peroxidase and polyphenoloxidase — according to D. M. Mikhlin and Ye. S. Branovitskaya [5].

During an examination of the data of Table 3, one can note the following. In most plants, there is weak acidification of the protoplasm by 0.1 - 0.3 and a slight increase in the oxidative-reduction potential under the effect of toxic gases. In the Norway maple, the acidifying effect of the gas was not noted, while in the rose — alkylation even occurs. More clearly defined changes occur with respect to the activity of the oxidative enzymes. As an enzyme carrying out an oxidative function, catalase sharply reduces its activity under the effect of the examined gases. An exception is the rose, in which the activity of catalase increases, and then decreases with a weak concentration of chlorine up to $2.5 \cdot 10^{-5}$%.

The activity of peroxidase and polyphenoloxidase also sharply drops when the gas enters. However, in oak leaves, the activity of peroxidase does not practically change as the result of fumigation of the leaves with SO_2, and even increases due to chlorine fumigation. In the ash, the activity of polyphenoloxidase increases more as the result of SO_2 striking the leaf. Hence, under the effect of toxic gases, a greater or lesser change in the activity of the oxidative-reduction systems occurs. An increase or a decrease in the activity of the iron-containing enzymes places in doubt the assertion of certain investigators that, in this case, cleavage of the iron occurs with inactivation of the enzymatic activity. It is not impossible that in this case the replacement of the effect of one enzymatic system with another occurs, if the disorders are not too profound. An increase in the intensity of respiration and a drop in the activity of the oxidative systems indicate the absence of coupling between these processes during the entry of the toxic gas into the leaf. In this case, unregulated destruction of substances occurs with the liberation of CO_2. The increase in the activity of

TABLE 3. CHANGE IN THE ACTIVITY OF ENZYMES, pH, AND rH_2 IN LEAVES AFTER THEIR FUMIGATION

Kind	Date	Gas and concentration	Activity of enzymes			pH, water	rH_2, water
			Catalase, ml 0.1 = H, H_2O_2	Peroxidase, mg Ag	Polyphenoloxidase, ml 0.01 = H, KJO_3		
Pedunculate oak	Sept. 6	Chlorine, $1 \cdot 10^{-4}$	32.29	17.10	5.5	5.7	15.1
		Sulfur dioxide	36.63	13.12	2.0	5.8	16.1
		Control	52.48	13.59	10.5	5.8	16.1
Norway maple	July 12	Chlorine	40.26	0.42	7.5	6.4	16.8
		Sulfur dioxide	35.87	0.66	0.0	6.4	16.8
		Control	79.99	0.84	0.5	6.4	16.8
Walnut	Sept. 3	Chlorine	69.62	-	7.0	5.0	14.4
		Sulfur dioxide	12.69	-	6.0	5.0	14.4
		Control	99.09	-	8.0	5.1	14.3
Chinese poplar	June 28	Chlorine	4.74	-	5.5	5.5	18.0
		Sulfur dioxide	16.79	-	0	5.7	17.9
		Control	73.95	-	20.9	5.9	17.9
Red poplar	Sept. 20	Chlorine, $1 \cdot 10^{-4}$	83.71	1.25	-	5.9	16.3
		Chlorine, $2.5 \cdot 10^{-5}$	116.23	-	-	6.0	16.3
		Control	120.92	5.0		6.1	16.3
White mulberry	July 19	Chlorine $1 \cdot 10^{-4}$	226.46	0.83	7.0	8.3	17.6
		" , $2.5 \cdot 10^{-5}$	235.74	0.67	9.5	8.3	17.4
		" , $1.7 \cdot 10^{-5}$	195.12	0.83	8.0	8.1	17.4
		Sulfur dioxide	116.93	0.67	5.5	7.8	17.3
		Control	169.67	0.83	48.5	8.1	17.2
Common ash	Sept. 7	Chlorine, $1 \cdot 10^{-4}$	35.69	15.67	40.0	5.6	15.7
		" , $2.5 \cdot 10^{-5}$	111.41	-	49.0	5.8	15.8
		Control	146.98	-	38.0	5.8	15.9

catalase and peroxidase is apparently due to blocking of the respiratory chain and an increase in the formation of peroxides.

With the goal of increasing the gas resistance of plants, a number of methods have been submitted and tested. Among them, methods providing for an improvement in conditions of root nutrition of the plants proved to be effective. Thus, in the experiments of N. P. Krasinskiy [3], introducing nitrate and ammonia fertilizers into the soil, particularly the former, increased the resistance of plants to the gas, while the phosphorous and potassium fertilizers had no positive effect. An increase in the resistance of plants to gases was achieved by introducing fertilizers when planting greenery at industrial enterprises.

Spraying plants with growth activating and other substances (β-naphthylacetic acid, 2,4-dichlorphenoxyacetic acid, indole, indolylacetic acid, tryptophane, isonicotinyl hydrazine, ascorbic acid, octadecene-1, and diphosphopyridinnucleotide) partially reduced, but did not eliminate, the injurious effect of gas on the plant [15, 16].

As already noted, plants which accumulate an increased amount of sulfur and chlorine in their organs and which have higher enzymatic system activity are more gas resistant. The plants we identified which were comparatively resistant to gases — such as the olive, rose, Turkestan poplar, oak, locust, and others — may simultaneously grow in saline soils. The nongas resistant species (linden, chestnut, etc.) have no such capacity. Consequently, there is common ground between the gas resistance and salt resistance of plants.

For plants themselves, the pathway of entry of the toxic substance does not have decisive significance — through the roots or through the leaves. As the experiments of Thomas showed [19], sulfur isotope $S^{35}O_2$ was included in the amino acids which were rapidly used to form proteins, independently of whether it penetrated through the leaves or through the roots. The latter was found in all organs of the plant.

TABLE 4. CHANGE IN pH, rH_2, ACTIVITY OF ENZYMES AND AMINO ACIDS FROM FUMIGATION OF SEEDLINGS WITH SULPHUR DIOXIDE AND CHLORINE

Watered with	Fumigation	pH water	rH_2 water	Activity of* 0.1 - n H_2S_4	Sum of free amino acids, T
		Walnut			
Control.	—	6.10	20,5	5[illegible]1	0,096
.	Sulfur dioxide	5,90	19,8	4[illegible]6	0,096
.	Chlorine	5,90	19,8	5,6	0,096
H_2SO_4	—	5,95	19,9	30,0	0,096
.	Sulfur dioxide	5,90	19,8	19,9	0,075
.	Chlorine	5,80	19,1	22,1	0,096
HCl	—	6,0	20,3	33,5	0,096
.	Sulfur dioxide	5,9	21,2	48,2	—
.	Chlorine	5,8	19,1	34,9	0,096
		Horse chestnut			
Control	—	6,55	21,0	15,1	0,166
.	Sulfur dioxide	6,15	21,2	10,2	0,192
.	Chlorine	6,10	21,0	11,0	0,192
H_2SO_4	—	6,30	20,9	28,4	0,166
.	Sulfur dioxide	6,20	21,0	15,1	0,166
HCl	—	6,35	21,0	31,5	0,166

*Translator's note. Illegible in original foreign text.

On the basis of the presented material, we hypothesized that one could increase the gas resistance of plants by means of growing them under conditions of an increased soil contant of those substances which are contained in the industrial gas discharges. To check this hypothesis, we soaked seeds of the walnut and horse chestnut in a 0.1% aqueous solution of sulfuric and hydrochloric acids. Then, the seeds were planted in pots filled with black earth, and were watered for a period of 2 months with a 0.02% solution of the same salts. The control was the same types of seeds which were soaked in distilled water, and watered with tap water. The plantings were grown in greenhouses in which a temperature of 22° C was maintained, and 16-hour illumination was created with DRL-250 bulbs.

Soaking the seeds and watering the seedlings with acid solutions affected the intensity of their growth and accumulation of the vegetative mass. The two-month-old walnut plants, during watering with sulfuric acid, had a height of 13 cm, with water — 10, and with hydrochloric acid — 8 cm. Consequently, sulfuric acid partially stimulated growth, while hydrochloric acid inhibited it.

In order to estimate the gas resistance of experimental and control seedlings, we fumigated them in polyethylene chambers with sulfur dioxide in a concentration of $15 \cdot 10^{-5}$ and chlorine in a concentration of $5 \cdot 10^{-5}$ % by volume.

Fumigation of the control plants with chlorine or sulfur dioxide causes necrosis of 10 - 20% (1 - 2 points) of the area of leaves, and in the experimental plants — no visible lesions are noted.

Calculation of the intensity of photosynthesis and respiration (Table 5) shows that watering seedlings of the walnut and chestnut with sulfuric acid reduces the rate of assimilation of carbon dioxide less than hydrochloric acid. This is in agreement with data of their growth. In a case of fumigating the plants, the strongest drop in assimilation of carbon dioxide is observed in the control plants, independent of the gas used.

During gassing with chlorine, the intensity of photosynthesis in seedlings watered with sulfuric acid dropped by 25 - 67%, while in those watered with hydrochloric acid, it dropped by 15 - 40%. During fumigation with sulfur dioxide in seedlings raised during watering with sulfuric acid, there was a drop in the intensity of photosynthesis in rare cases, but more often there was an increase, while in those watered with hydrochloric acid, the decrease in assimilation of carbon dioxide was greater.

On the day after fumigation, the intensity of photosynthesis usually reaches values near its level before gassing in the uninjured leaves.

The effect of fumigation increases the intensity of respiration slightly, but the value of the increase is greater in the control leaves. Watering walnut seedlings with acid reduces the pH of cellular contents by 0.2 - 0.3, and simultaneously reduces the oxyredox potential toward greater reduction of substances (see Table 3).

TABLE 5. INTENSITY OF PHOTOSYNTHESIS AND RESPIRATION OF SEEDLINGS BEFORE AND AFTER FUMIGATING THEM WITH CHLORINE AND SULFUR DIOXIDE (in mg CO_2 per 1 dm^2 per hour)

Experiment variation	Watered with	Fumigated with	Photosynth. intensity		Respiration intensity	
			Before	After	Before	After
Walnut	Control	Chlorine	18,6	4,6	1,5	2,0
	H_2SO_4		17,4	5,4	2,2	3,8
	HCl		16,2	12,4	1,6	2,0
	Control		21,4	9,4	—	—
	H_2SO_4		17,1	11,8	—	—
	HCl		16,3	10,7	—	—
Horse chestnut	Control		18,3	0,69	1,8	6,9
	H_2SO_4		11,1	3,7	1,1	3,7
	HCl		7,5	6,4	1,6	2,1
	Control		7,6	2,1	—	—
	H_2SO_4		8,7	5,5	—	—
	HCl		10,5	7,0	—	—
Walnut	Control	Sulfur dioxide	11,8	7,9	—	—
	H_2SO_4		16,1	29,1	—	—
	HCl		10,4	9,2	—	—
	Control		15,9	17,0	—	—
	H_2SO_4		21,6	31,3	—	—
	HCl		18,5	9,3	—	—
Horse chestnut	Control		14,8	7,0	—	—

Additionally, the action of acids in walnuts causes a drop in the activity of catalase by almost twofold, while in the chestnut — to the contrary, it causes an increase of the same magnitude.

In the case of fumigating the experimental and test plants with gases, acidification of the cytoplasm and a drop in the activity of catalase occurs, to a greater degree from Cl_2 exposure than from exposure to SO_2.

Watering walnut seedlings with sulfuric acid with subsequent sulfur dioxide fumigation facilitates a drop in the total amount of free amino acids. This may be explained by their accelerated utilization for protein synthesis. No such changes occur in the leaves of the chestnut. Moreover, the control seedlings have twice as much amino acid, due to a reduction in the rate of their involvement in the basal metabolism.

Hence, the data we obtained give basis to the conclusion that raising plants under conditions of an increased content of anions in the soil solution causes adaptive reactions to them in the organism. In case of their entry into a polluted atmosphere, such plants are less damaged and processes of their vital activity are less disrupted. Therefore, for the practice of planting greenery at industrial enterprises, it is expedient to use the seeds of plants grown in weakly and moderately saline soils containing chloride-sulfate salts, with subsequent raising of seedlings in the same soils. It is permissible to create artificially salted soils and to grow seedlings from ordinary seeds in them.

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